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Enantioselective sensing of amino acids by copper(II) complexes of phenylalanine-based fluorescent β-cyclodextrins

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Abstract

The synthesis and full characterisation of two modified cyclodextrins 6-deoxy-6-*N*-[N^{α}-(N^{2}-dansylaminoethyl)-*R*-(or *S*)-phenylalaninamide]-β-cyclodextrin, containing a metal binding site and a dansyl fluorophore, are described. Both cyclodextrins were shown to form copper(II) complexes with fluorescence quenching. Addition of D- or L-amino acids to the copper(II) complexes induced a 'switch on' of the fluorescence which was enantioselective for Pro, Phe and Trp. The enantioselective fluorescence effect was used for the determination of the optical purity of proline. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: fluorescence; cyclodextrins; sensors; amino acids; enantioselection.

Fluorescent sensors based on molecular recognition are of particular interest, on account of their high sensitivity and selectivity,[1] being able to detect metal ions,[2] anions[3] and organic bioactive molecules.[4]

Modified cyclodextrins bearing fluorescent moieties have been used by Ueno and co-workers[5] as sensors for a wide variety of organic guests, since they allow improvement of solubility in water, enhancement of fluorophore quantum yield, and use of the lipophilic cavity as a recognition motif.

Enantioselectivity is one of the most important goals in developing sensors for organic analytes,[6] biological activity in most cases being related to stereochemistry. However, very few examples of enantioselective fluorescence sensors have been reported so far.[7,8]

The synthesis and complexing properties of modified β-cyclodextrins bearing binding sites for copper(II) and a dansyl fluorophore, 6-deoxy-6-*N*-(*N*′-dansylethylenediamino)-β-cyclodextrin (CD-en-DNS†) and 6-deoxy-6-*N*-(*N*′′-dansyldiethylenetriamino)-β-cyclodextrin (CD-dien-DNS) have recently been described.[9,10] In contrast with CD-en-DNS, the cyclodextrin CD-dien-DNS was found to undergo fluorescence quenching when forming a complex with copper(II); by addition of amino acids to the

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† Abbreviations. DNS: dansyl (5-dimethylaminonaphthalene-1-sulfonyl); Boc: *tert*-butoxycarbonyl; HBTU: *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; DIEA: *N*,*N*-diisopropylethylamine.

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copper(II) complex, the fluorescence was ‘switched on’ due to the formation of ternary complexes, by displacement from copper(II) of the weakest binding site (the sulfonamide of the dansyl group).[10] However, no enantioselectivity was observed.

In order to obtain enantioselective fluorescence sensors for unmodified amino acids, we report in the present work the design and synthesis of two new cyclodextrins 6-deoxy-6-*N*-[N^{α}-(*N*^{2}-dansylaminoethyl)-*R*-(or *S*)-phenylalaninamide]-β-cyclodextrin **(*R*)-1** and **(*S*)-1** (Fig. 1), in which a stereogenic centre (*R* or *S*) has been introduced in the side-arm. The design is based on our previous experience on enantiomeric separation by copper(II) complexes of chiral ligands[11] or of modified cyclodextrins[12] (chiral ligand exchange chromatography).

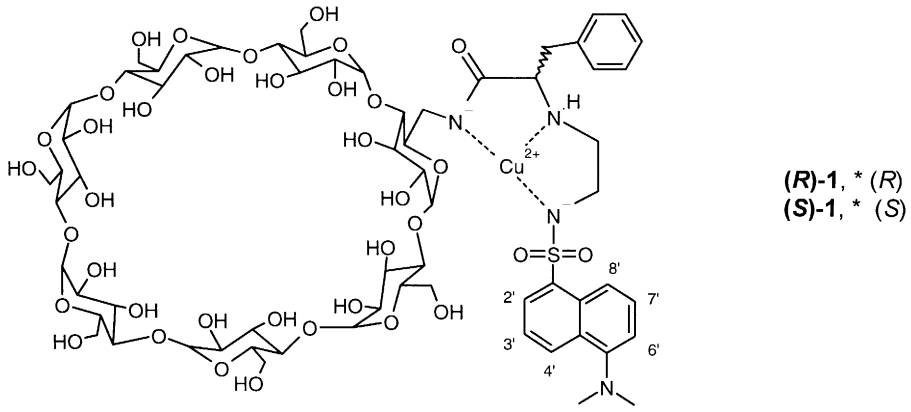

Fig. 1. Schematic structure of the copper(II) complexes of dansylated cyclodextrins **(*R*)-1** and **(*S*)-1**

The new cyclodextrins have three binding sites for copper(II): an amide, an amino and a sulfonamide group, and were synthesised as follows. N^{α}-(*N*^{2}-Boc-aminoethyl)-*R*-(or *S*)-phenylalanine benzyl ester (*R*)-**2** and (*S*)-**2** were synthesised from (*R*)- and (*S*)-phenylalanine benzyl ester as reported in the literature.[13] By simultaneous deprotection of the Boc and benzyl groups by $AlCl_3$ and subsequent reaction with dansyl chloride at low temperature (0°C), N^{α}-(*N*^{2}-dansylaminoethyl)-*R*-(or *S*)-phenylalanine (*R*)-**3** or (*S*)-**3** were obtained, avoiding sulfonylation of the secondary amine. The cyclodextrins **(*R*)-1** and **(*S*)-1** were then obtained by reaction of 6-deoxy-6-amino-β-cyclodextrin[14] with (*R*)-**3** or (*S*)-**3**, respectively, in the presence of HBTU as condensing agent (Fig. 2). The products were purified by HPLC as trifluoroacetate salts and fully characterised, as reported below.

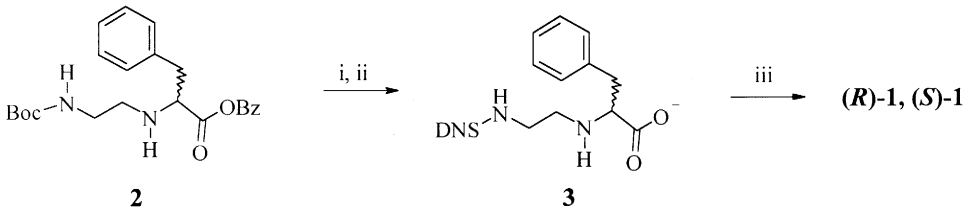

Fig. 2. Synthesis of modified cyclodextrins **(*R*)-1** and **(*S*)-1**: (i) $AlCl_3$, anisole, CH_3NO_2, CH_2Cl_2, 0°C, 15 min; (ii) dansyl chloride, Li_2CO_3, H_2O, CH_3CN, 0°C, 4 h; (iii) 6-deoxy-6-amino-β-cyclodextrin, HBTU, DIEA, DMF dry, 3 h

Both cyclodextrins were quenched by copper(II) at pH=7.3 forming 1:1 complexes (**Cu–(*R*)-1** and **Cu–(*S*)-1**). However, the fluorescence quenching was different for the two cyclodextrins, suggesting that a weaker complex was formed with **(*R*)-1** (results not shown).

By addition of D- and L-amino acids to the copper(II) complexes **Cu–(*R*)-1** and **Cu–(*S*)-1** in a 1:1 ratio, an increase of the fluorescence was observed (Fig. 3), depending on the type of amino acid used and, in some cases, on its absolute configuration.

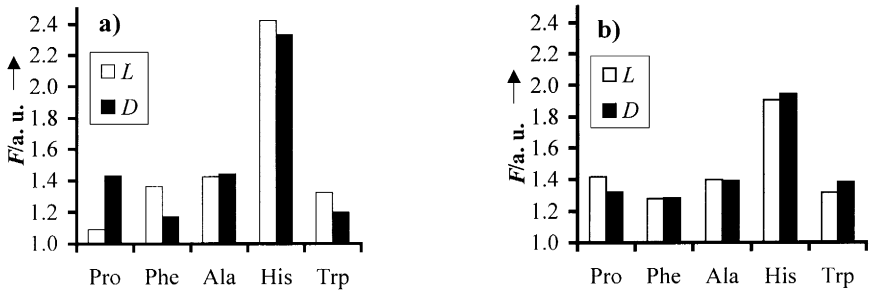

Fig. 3. Fluorescence increase of (a) **Cu–(*S*)-1**, (b) **Cu–(*R*)-1** (6×10^{-5} M) upon addition of equimolar amount of D- or L-amino acids in 0.1 M tetraborate buffer (pH=7.3)

The enantioselectivity in the fluorescence 'switch on' can be due to the formation of ternary cyclodextrin:copper(II):amino acid complexes of different stabilities, with displacement of the dansyl group from copper(II).

The complex **Cu–(*S*)-1** (Fig. 3a) showed better discriminating properties than **Cu–(*R*)-1** (Fig. 3b). The best enantioselectivity was observed for proline with both cyclodextrins, with reversed order ($\Delta F_D/\Delta F_L$=3.89 for **Cu–(*S*)-1** and $\Delta F_D/\Delta F_L$=0.74 for **Cu–(*R*)-1**).

For aromatic amino acids (phenylalanine and tryptophan) a significant enantiomeric differentiation was observed with **Cu–(*S*)-1**, whereas for alanine, which bears a small aliphatic side chain, a negligible difference was found between the two enantiomers. This suggests a possible involvement of the cyclodextrin cavity in complex formation. Enantioselectivity for Pro was the reverse of that observed for Phe ($\Delta F_D/\Delta F_L$=0.33) and Trp ($\Delta F_D/\Delta F_L$=0.63).

In the case of His, a large fluorescence increase was observed for both enantiomers, on account of its strong histamine-like (amino and imidazolyl groups) binding sites.

Titration experiments, performed with **Cu–(*S*)-1** and with both Pro and Phe, showed an enantioselective increase of the fluorescence intensity (Fig. 4).

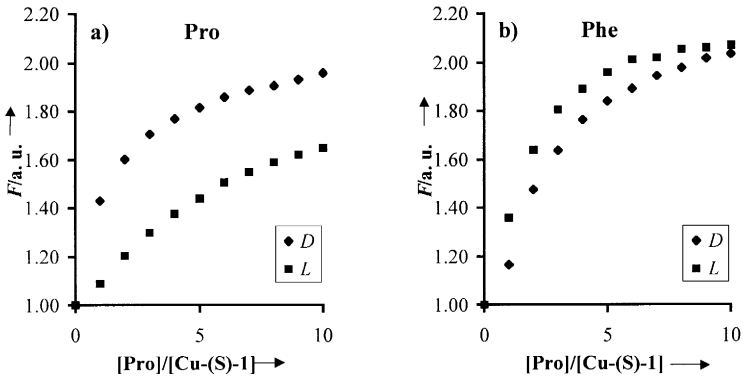

Fig. 4. Titration curves of **Cu–(*S*)-1** (6×10^{-5} M) with (a) L-Pro (■) and D-Pro (◆); (b) L-Phe (■) and D-Phe (◆), in 0.1 M tetraborate buffer (pH=7.3)

A significant difference was observed upon adding up to a 10-fold excess of Pro, whereas in the presence of excess Phe both enantiomers converged to the same fluorescence value. Therefore, the

difference between the stabilities of the diastereomeric ternary complexes of **Cu–(*S*)-1** with proline is higher than with phenylalanine.

The recognition mechanism is still under investigation, and experiments are being carried out in order to gain evidence on the structure of the ternary complexes and on the fluorescence 'switch on' process, in particular on the involvement of the cyclodextrin cavity in complexation.

Cu–(*S*)-1 can be considered as an enantioselective fluorescence sensor for Pro in the concentration range analysed (6×10^{-5}–6×10^{-4} M). It was used (6×10^{-5} M, pH=7.3) for the determination of the optical purity of proline in dilute solutions (6×10^{-4} M, 0.1 mg total amount). Calibration was performed with enantiomerically pure D- and L-proline, as reported in Fig. 3a. Three unknown solutions (A, B, C) were analysed by measuring the fluorescence intensity in the presence of **Cu–(*S*)-1** under the conditions mentioned above, by means of the expression: D/(D+L)%=100 $(F_i\text{-}F_L)/(F_D\text{-}F_L)$ where F_i is the fluorescence intensity of the i^{th} sample and F_D and F_L are those of the pure enantiomers. The results were compared with the actual values obtained by weight and by GC.‡ D/(D+L)% (actual values): A: 15%, B: 60%, C: 70%; D/(D+L)% (measured by fluorescence) A: 19% (s.d.=2), B: 58% (s.d.=2), C: 70% (s.d.=3). It should be noted that the detection of D-proline is particularly interesting, since this enantiomer has been found in foods,[15] and has given rise to controversial reports concerning its toxicity.[16]

We think that the present method is quite promising, since it does not require amino acid derivatisation (as in GC), it can be performed on a very low amount of sample and has a better or at least similar accuracy compared to other spectroscopic methods such as NMR (using chiral shift reagents).

Spectral data. **(*R*)-1** Yield 22%. Mp 206°C (dec.); UV–vis (H_2O): λ_{max} (ε)=247 (16430), 332 (4770); ^{1}H NMR (300 MHz, D_2O, 25°C): δ=2.85–3.00 (m, 2H), 3.05–3.15 (m, 1H), 3.25–4.13 (m, 50H), 4.19 (m, 1H), 4.39 (dd, 1H, ^3J=10.1, 5.2 Hz, N-CH-CO), 4.97 (d, 1H, ^3J=3.3 Hz, H_1), 5.07 (d, 1H, ^3J=3.6 Hz, H_1), 5.11 (d, 1H, ^3J=3.4 Hz, H_1), 5.16–5.23 (m, 4H, H_1), 7.25 (d, 2H, ^3J=6.6 Hz, H_{Ph}), 7.50–7.57 (m, 3H, H_{Ph}), 8.03–8.08 (m, 2H, $H_{3',7'}$), 8.22 (d, 1H, ^3J=7.7 Hz, $H_{6'}$), 8.52 (d, 1H, ^3J=7.4 Hz, $H_{2'}$), 8.59 (d, 1H, ^3J=8.7 Hz, $H_{4'}$), 8.90 (d, 1H, ^3J=8.7 Hz, $H_{8'}$) ppm; IR (KBr): 3650–3050 (O–H, N–H), 1650 (C=O), 1150 (S=O), 1032 (C–O) cm^{-1}; MS (ESI): *m/z* (%): 1558 (100) [M+H$^+$]. Anal. calcd for $C_{69}H_{98}N_4O_{41}SF_6\cdot1H_2O$: C, 45.95; H, 5.59; N, 3.11. Found: C, 45.87; H, 5.28; N, 3.23.

(*S*)-1: Yield 29%. Mp 200°C (dec.); UV–vis (H_2O): λ_{max} (ε)=248 (14450), 332 (3870); ^{1}H NMR (300 MHz, D_2O, 25°C): δ=3.20–4.05 (m, 53H), 4.15 (m, 1H), 4.33 (m, 1H, N-CH-CO), 5.05–5.20 (m, 7H; H_1), 7.30–7.34 (m, 2H; H_{Ph}), 7.50–7.60 (m, 3H; H_{Ph}), 8.06–8.12 (m, 2H; $H_{3'}$, $H_{7'}$), 8.27 (d, ^3J=7.4 Hz, 1H; $H_{6'}$), 8.46 (d, ^3J=7.7 Hz, 1H; $H_{8'}$), 8.64 (d, ^3J=8.7 Hz, 1H; $H_{2'}$), 8.93 (d, ^3J=8.8 Hz, 1H; $H_{4'}$); IR (KBr): 3650–3050 (O–H, N–H), 1683 (C=O), 1150 (S=O), 1025 (C–O) cm^{-1}; MS (ESI): *m/z* (%): 1558 (100) [M+H$^+$], 798 (65) [*M*+H$^+$+K$^+$], 779 (83) [M+2H$^+$]. Anal. calcd for $C_{67}H_{97}N_4O_{39}SF_3\cdot15H_2O$: C, 41.44; H, 6.59; N, 2.89. Found: C, 41.42; H, 6.82; N, 3.03.

Acknowledgements

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‡ Trifluoroacetyl proline isopropyl esters were analysed with a 2,6-di-*O*-methyl-3-trifluoroacetyl-γ-cyclodextrin column with the following temperature program: 90°C (2 min), 90–150°C (4°/min), 150°C (15 min). The peaks of the two enantiomers were baseline separated.

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